#19
M.M.

PATENT 10/00/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Philip E. Thorpe and Sophia Ran	§	
	§	Group Art Unit: 1619
Serial No.: 09/351,862	§	
	§	Examiner: Sharareh, S.
Filed: July 12, 1999	§	Atty. Dkt.: 4001.002282
For: CANCER TREATMENT KITS USING ANTIBODIES TO AMINOPHOSPHOLIPIDS	§	

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

September 21, 2001
Date

Shelley P.M. Fussey

DECLARATION OF PHILIP E. THORPE AND SOPHIA RAN UNDER 37 C.F.R. § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

WE, PHILIP E. THORPE AND SOPHIA RAN, HEREBY DECLARE AS FOLLOWS:

1. We are co-inventors of the subject matter disclosed and claimed in the captioned patent application.

2. I, Dr. Philip E. Thorpe, am Professor of Pharmacology and hold the Serena S. Simmons Distinguished Chair in Immunopharmacology at the Simmons Cancer Center, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas ("UT Southwestern"). I am a

British subject and a permanent resident in the United States. I live at 5311 Nakoma Drive, Dallas, Texas, 75209, U.S.A.

3. I, Sophia Ran, am an Assistant Professor at the Simmons Cancer Center, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas. I am a citizen of Israel and a permanent resident in the United States. I live at 5840 Spring Valley Road, #1612, Dallas, Texas, 75240, U.S.A.

4. We have reviewed the Official Action issued May 08, 2001 by the U.S. Patent and Trademark Office (P.T.O.), which is charged with assessing the patentability of the captioned patent application. We have also reviewed the references cited in the Official Action: U.S. Patent No. 6,197,278 to Blankenberg, Huang *et al.*, *Science*, 275:547-550, 1997; WO 98/29453; and Fishman *et al.*, *Intl. J. Oncol.*, 10:901-904, 1997.

5. We understand that the P.T.O. has taken the position that the claims examined in the captioned patent application would be obvious to one of skill in this field of study in light of U.S. Patent No. 6,197,278 in view of Huang *et al.*, 1997, WO 98/29453 and Fishman *et al.*, 1997.

6. We disagree with the assessment that the referenced combination of documents would render the presently claimed subject matter obvious to a scientist working in this field of research.

7. According to the cover page of the document itself, we understand that WO 98/29453 was published on July 09, 1998.

8. We are providing the present declaration and attached documentary evidence to demonstrate that the invention claimed in the captioned patent application was made in the United States prior to July 09, 1998, *i.e.*, prior to the publication date of the WO 98/29453 document.

9. Evidence of the fact that the invention claimed in the captioned patent application was made in the United States prior to July 09, 1998 is shown in the attached Exhibits and described in the following paragraphs. The studies described in the following paragraphs were conducted in Dallas, Texas, in the United States.

10. The captioned patent application claims kits that comprise at least a first antibody, or an antigen-binding fragment thereof, that binds to an aminophospholipid expressed on tumor blood vessels. In these kits, the anti-aminophospholipid antibody is combined with either a detectably-labeled antibody, or antigen-binding fragment thereof, that also binds to an aminophospholipid, or with an anti-cancer agent.

11. The anti-aminophospholipid antibodies of the kits in the captioned patent application are "naked" or unconjugated antibodies. The anti-aminophospholipid antibodies include those that bind to the aminophospholipid, phosphatidylserine (PS), and those that bind to the aminophospholipid, phosphatidylethanolamine (PE). As described in the specification of the

captioned patent application, we discovered that naked antibodies against aminophospholipid components are capable of specifically inducing tumor blood vessel destruction and tumor necrosis *in vivo*, and exert such tumor destructive effects in the absence of conjugation to effector molecules, such as toxins or coagulants.

12. Exemplary evidence of the concept of the claimed invention is provided in **Exhibit A**, a copy of an Invention Disclosure that we submitted to the Office of Legal Affairs and Technology Transfer at UT Southwestern prior to July 09, 1998.

13. The Invention Disclosure of **Exhibit A**, submitted prior to July 09, 1998, describes the discovery that antibodies to phosphatidylserine localize specifically to tumor vascular endothelium after injection into tumor bearing mice. It is stated that the antibodies induce thrombosis of tumor blood vessels, followed by tumor regression and that the antibodies produce anti-tumor effects in mice with no evidence of toxicity. It is further explained that this type of antibody is useful for therapy of numerous types of solid tumors, since PS exposure on the endothelium should be common to different tumor types.

14. In the Invention Disclosure of **Exhibit A**, we particularly explain that this invention differs from the use of antibodies to target toxins or coagulants to tumor vasculature, as a linked effector molecule is not required for activity. We mention some possible mechanisms underlying the anti-tumor effects of the invention, including inducing apoptosis of tumor vascular endothelial cells and initiating complement-mediated lysis of such endothelial cells.

15. Data showing the use of naked anti-aminophospholipid antibodies to successfully treat tumors *in vivo* prior to July 09, 1998 is shown in **Exhibit B**. This exhibit is a copy of materials that I, Sophia Ran, used in a confidential presentation to members of our laboratory. The design of the studies involves the injection of anti-PS and control antibodies (mIgM) into tumor-bearing mice and determining the localization of the administered antibodies on tumor vessels and normal vasculature. The treatment of tumor-bearing animals is exemplified by the treatment of L540 Hodgkin's tumor-bearing SCID mice with two intravenous injections of anti-PS or control antibodies (20 μg/dose, with a 48 hour interval).

16. **Exhibit B** includes a table and figure of the data generated from the treatment of L540 Hodgkin's tumor-bearing mice tumor in studies conducted prior to July 09, 1998. The administration of anti-PS antibodies to these tumor-bearing mice is shown to result in anti-tumor effects in comparison to the control antibody. These studies are summarized as showing that anti-PS IgM, but not control IgM, specifically localized to tumor blood vessels in the treated animals, and that no localization was observed to blood vessels of normal organs. It is further stated that some of the anti-PS-injected L540-bearing mice developed thrombosis and even necrosis 4-24 hours post treatment, whereas none of the controls or untreated mice showed thrombosis or necrosis (**Exhibit B**).

17. Additional evidence of the development of this invention prior to July 09, 1998 is shown in **Exhibit C**, a copy of correspondence from Philip E. Thorpe to Mr. Richard U. Rodriguez of the Office of Legal Affairs and Technology Transfer at UT Southwestern. This correspondence

documents the shipment of antibodies against phosphatidylserine from Dr. Neal Rote of Wright State University to Drs. Thorpe and Ran at a date prior to July 09, 1998 (**Exhibit C**). This correspondence repeats the summary Dr. Ran's observations that administration of an anti-phosphatidylserine antibody to tumor bearing mice caused selective thrombosis of tumor blood vessels.

18. Evidence of the progress of this invention into a patent application is shown in **Exhibit D**, a copy of correspondence from Mr. Ray Wheatley, Director of Technology Transfer in the Office of Legal Affairs and Technology at UT Southwestern, to Mr. Louis Pirkey of the law firm of Arnold, White & Durkee. The correspondence from Mr. Wheatley references our invention disclosure (**Exhibit A**) and asks that the matter be handled by Shelley Fussey, then employed at Arnold, White & Durkee. The correspondence of **Exhibit D** also includes the particular file code "UTSD:549", which still forms the basis of UT Southwestern's file reference for the captioned application (UTSD:549--1).

19. We recall that a lengthy and detailed draft of the provisional application was prepared by Shelley Fussey and forwarded for our review prior to July 09, 1998. I, Philip E. Thorpe, particularly recall meeting with Shelley Fussey, who traveled to Dallas to discuss the near-to-final draft of the application prior to July 09, 1998, and I have recorded this meeting on my calendar for the date in question.

20. From the time of our documented development of the invention prior to July 09, 1998, we worked diligently on various aspects of the invention in the United States up to and including

July 13, 1998, when the first U.S. provisional patent application directed to our invention was filed.

21. **Exhibit E** shows front page, claims and abstract of the first U.S. provisional patent application directed to our invention that was filed on July 13, 1998. The claims include antibodies that bind to phosphatidylserine, as represented at least in claims 3, 6, 9, 12, 15, 83, 86, 89 and 93; and claims embodying various possible mechanisms underlying the anti-tumor effects of the invention, as represented at least in claims 1, 4, 7, 10, 13, 75-81, 84 and 87, including the possible apoptosis and complement-mediated lysis mechanisms (claims 77 and 78) discussed in our Invention Disclosure (**Exhibit A**). Kits comprising the naked anti-aminophospholipid antibodies of the invention are particularly represented by claims 91-100.

22. From a time prior to July 09, 1998, through July 13, 1998 to the present time, we have continued to work diligently on various aspects of the claimed invention in the United States.

23. We hereby declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9/20/01	Philip Thorpe
Date	Philip E. Thorpe
9/20/01	Sophia Ran.
Date	Sophia Ran

THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER
INTELLECTUAL PROPERTY QUESTIONNAIRE

DESCRIPTION OF THE INVENTION

Descriptive Title of Invention: Immunotherapy of cancer with antibodies to a marker of tumor vascular endothelium

Who are the individuals that contributed to the conception of the invention (attach Inventor Information Page for each):

Philip E. Thorpe
Sophia Ran

INTELLECTUAL PROPERTY DESCRIPTION

(Attach separate pages, if necessary)

Briefly summarize the invention, its use and purpose: We have discovered that IgM antibodies to phosphatidyl serine (PS) localize specifically to tumor vascular endothelium after injection into tumor-bearing mice. The antibodies induce thrombosis of tumor blood vessels followed by tumor regression.

What particular features of the invention are unusual? 1) The antibodies should be useful for therapy of numerous types of solid tumors, since PS exposure on the endothelium should be common to different tumor types, and may, in fact, be the primary reason why tumor vasculature is prothrombotic. 2) The antibody has, thus far, produced anti-tumor effects in mice with no evidence of toxicity.

How does it differ from present technology? We have already filed patents/patent applications describing the use of antibodies to tumor vasculature markers for targeting toxins, coagulants etc. to tumor vasculature. The current invention differs from this earlier work in not requiring a linked effector molecule for activity. The anti-PS antibodies probably act either by inducing apoptosis of tumor vascular endothelial cells or by initiating complement-mediated lysis of the endothelial cells.

What problem(s) does it solve? The anti-PS antibodies may not need to gain access to tumor cells themselves for activity, since they bind to the accessible tumor endothelium. Also, the approach should be generally applicable to solid tumors.

What advantages over current technologies does it possess?

Potentially lower toxicity
Simplicity
Ease of manufacture for clinical use

CONFIDENTIAL

Type of intellectual property: (Circle all that apply)

(Biological material) Computer Software
Device Copyrightable Work
Other: _____

If biological material is incorporated or was used in the research leading to the invention, did your research utilize biological material obtained from some source other than your laboratory (circle):

(Yes) No If yes, what is the source? Dr. Neal Rote, Dept. of Microbiology and Immunology, Wright State University, Dayton, Ohio

Please attach any written agreement (e.g. letter, agreement) pertaining to the use of the material.

Please categorize the potential uses of your invention below by circling all anticipated uses:

Service
(Product-Therapeutic)
Product-Device
Product-Method
Research Reagent
Drug Identification
Diagnostic Test
Other: _____

Identify the diseases or condition affected by the invention:

Indication	Patients in U.S./ Year	New Cases in U.S./ Year	Deaths in U.S./ Year
Cancer		1,200,000	500,000

What companies do you believe would be interested in commercializing the invention?

Many

INVENTOR INFORMATION

Attach one page for each inventor named on the first page.

Inventor's Name: Thorpe (Last) Philip (First) E. (MI) Ph.D. (Degree)

Social Security #: 452 - 99 - 7852 U.S. Citizen (circle)? Yes (No)

Department: Pharmacology Division: _____ Campus Mail Station: 8593

Campus Phone #: 81275 Campus FAX #: 81266

E-mail Address: thorpe01@utsw.swmed.edu

Position Title (circle): (Professor) Assoc. Professor Asst. Professor Instructor Fellow Student Resident Resident Staff Other: _____

Other Appointment (circle): None HHMI VA Other: _____

% of time employed at UT: 100 % Other employment (circle one)? Yes No

(If yes, name of employer: _____)

Home Address: 6918 Westlake Avenue

Dallas, TX 75214

City State ZIP

Home Phone #: 214-324-0010

If a patent application for the intellectual property disclosed above is filed in the U.S. Patent and Trademark Office, the inventor(s) will be required to execute an oath or declaration affirming, among other things, that he/she/they is/are the original and first inventor(s) of the subject matter claimed in the application. ("Original" means derived from any source or persons other than the person or persons named as the inventorship entity.) I/We confirm that the subject matter disclosed herein is original to me/us.

Inventor's Signature: Philip E. Thorpe

Typed/Printed Name: Philip E. Thorpe Date: _____

Please attach a copy of your current C.V.

INVENTOR INFORMATION

Attach one page for each inventor named on the first page.

Inventor's Name: Ran (Last) Sophia (First) (MI) Ph.D. (Degree)

Social Security #: 423 - 31 - 0407 U.S. Citizen (circle)? Yes (No)

Department: Pharmacology Division: _____ Campus Mail Station: 8593

Campus Phone #: 81275 Campus FAX #: 81266

E-mail Address: _____

Position Title (circle): Professor, ~~Assoc. Professor~~, (Asst. Professor), Instructor, Fellow, Student, Resident, Resident, Staff, Other: _____

Other Appointment (circle): None HHMI VA Other: _____

% of time employed at UT: 100 % Other employment (circle one)? Yes No

(If yes, name of employer: _____)

Home Address: 202 Dogwood

Plano, TX 75075

(City) (State) (ZIP)

Home Phone #: 972-509-1610

If a patent application for the intellectual property disclosed above is filed in the U.S. Patent and Trademark Office, the inventor(s) will be required to execute an oath or declaration affirming, among other things, that he/she/they is/are the original and first inventor(s) of the subject matter claimed in the application. ("Original" means derived from any source or persons other than the person or persons named as the inventorship entity.) I/We confirm that the subject matter disclosed herein is original to me/us.

Inventor's Signature: Sophia Ran

Typed/Printed Name: Sophia Ran Date: _____

Please attach a copy of your current C.V.

Experimental design:

- Inject anti PS Ab and control Ab (mIgM from N.Rote) into tumor-bearing mice

- Determine localization of mIgM on tumor vessels and normal vasculature

Summary of anti PS IgM localization studies:

1) Anti PS IgM, but not control IgM, specifically localized to tumor blood vessels of L540 Hodgkin's tumor, HT29 (human colon adenocarcinoma) and H358 (human lung carcinoma).

2) No localization was observed to blood vessels of normal organs

3) Localization was tumor size-dependent, no homing of Ab was observed in small tumors below 300 ul. This is consistent with the observation that small L540 tumors do not respond to anti VCAM-tTF treatment.

4) Some of anti PS - injected L540 bearing mice developed thrombosis and even necrosis 4 -24 hours post treatment. None of the controls or untreated mice showed thrombosis or necrosis.

Summary of exps designed to test this hypothesis

1. Treatment of L540 Hodgkin bearing scid mice with 20 ug/dose of anti PS TC sup i.v., 2 injections with 48 hours interval

2 treated vs 2 controls

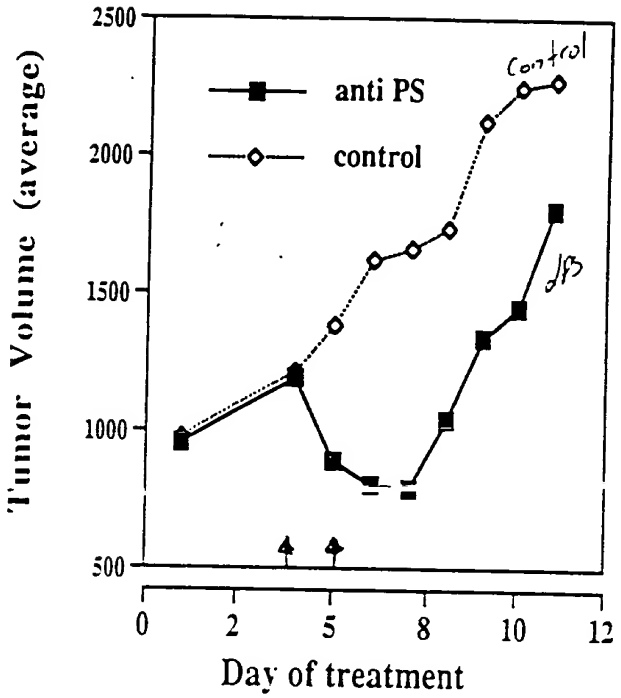
Effect of anti PS TC sup on growth of L540 tumors						
Date	1	2		3	4	
	Anti PS	Anti PS	Average	Control	Control	Average
	787	320	**553.5**	787	627	**707**
	972	600	**786**	936	936	**936**
	1050	860	**955**	940	1014	**977**
	1460	**924**	**1192**	**1190**	**1240**	**1215**
	1054	726	**890**	1386	1380	**1383**
	856	**750**	**803**	**1520**	**1720**	**1620**
	920	660	**790**	1680	1640	**1660**
	1240	840	**1040**	1790	1680	**1735**
	1570	1110	**1340**	2300	1950	**2125**
	1600	1240	**1450**	2400	2100	**2250**
	2040	1570	**1805**	2350	2200	**2275**
Mice sacrificied on						
Days of injections shown in bold						
Mouse number 1 developed thrombosis after first injection						

Effect of anti PS TC sup on growth of L540 tumor in scid mice

Sophia

Linda

Watkins



2 injections

48 h

interval

SOUTHWESTERN
THE UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER
AT DALLAS

Philip E. Thorpe, Ph.D.
Professor
The Serena S. Simmons Distinguished Chair
in Cancer Immunopharmacology

Department of Pharmacology
Nancy B. and Jake L. Hamon Center
for Therapeutic Oncology Research

Mr. Richard U. Rodriguez
Technology Analyst
Office of Legal Affairs and
Technology Transfer
UT Southwestern

RE: UTSD:549 – Immunotherapy of Cancer with Antibodies to a Marker of Tumor Vascular Endothelium

Dear Mr. Rodriguez:

The monoclonal antibodies, 35B9b against phosphatidylserine and D11A4 against cardiolipin, were sent to Dr. Sophia Ran and myself by Dr. Neal Rote of Wright State University, Ohio, without any commercial obligations or material transfer agreement. We agreed to include Dr. Rote as a coauthor on primary manuscripts but this is the only obligation of which we are aware.

The first shipment of antibodies was made on . The idea of using the antibodies for immunotherapy that is the subject of the above invention came from the surprising observation by Dr. Ran that administration of Dr. Rote's anti-phosphatidylserine antibody to tumor bearing mice caused selective thrombosis of tumor blood vessels.

Yours sincerely,

Philip Thorpe

Philip E. Thorpe, Ph.D.
Professor of Pharmacology

cc: Dr. Sophia Ran
Mr. Ray Wheatley
Ms. Shelley Fussey

THE UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER
AT DALLAS

Ray Wheatley, M.S.
Director of Technology Transfer

Office of Legal Affairs
and Technology Transfer

Louis T. Pirkey, Esq.
Arnold, White & Durkee
1900 One American Center
600 Congress Avenue
Austin, TX 78701-3248

Re: Authorization for Oral Patentability Search & Opinion for UTSD:549 — "Immunotherapy of Cancer with Antibodies to a Marker of Tumor Vascular Endothelium"

Dear Mr. Pirkey:

I hereby authorize an oral patentability search and opinion for the above referenced technology. I would request this file be handled by Shelley Fussey, Ph.D. If the search results are positive, I would like a provisional patent application filed under the fixed-fee filing system.

I have enclosed a copy of the technology disclosure and supporting documentation. If you require additional information, please contact Richard Rodriguez at 214-648-1884 or the inventors.

If you have any questions, please contact me.

Sincerely,

Ray Wheatley

Enclosures

cc: Shelley Fussey, Ph.D. (w/ enclosures)
Beth Lynn Maxwell, Ph.D., J.D. (w/ enclosures)
Sophia Ran, Ph.D. (w/o enclosures)
Philip E. Thorpe, Ph.D. (w/o enclosures)

5323 Harry Hines Blvd. / Dallas, Texas 75235-9008 / (214)648-3290 FAX (214)648-3675
E-mail: rwheat@mednet.swmed.edu

PATENT

UTSD:549PZ1

PROVISIONAL PATENT APPLICATION

for

CANCER TREATMENT USING ANTIBODIES TO AMINOPHOSPHOLIPIDS

by

Philip E. Thorpe

and

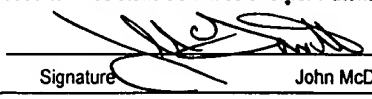
Sophia Ran

EXPRESS MAIL MAILING LABEL

NUMBER EM 545 970 255 US

DATE OF DEPOSIT July 13, 1998

I hereby certify that this paper or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service on the date indicated above and is addressed to: Assistant Commissioner for Patents, Washington D.C. 20231.



Signature John McDavitt

WHAT IS CLAIMED IS:

1. A method for killing tumor vascular endothelial cells, comprising administering to an animal having a vascularized tumor a biologically effective amount of an antibody, or antigen-binding region thereof, that binds to an aminophospholipid on the luminal surface of tumor vascular endothelial cells.

2. The method of claim 1, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylethanolamine on the luminal surface of tumor vascular endothelial cells.

3. The method of claim 1, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylserine on the luminal surface of tumor vascular endothelial cells.

4. A method for inducing coagulation in tumor vasculature, comprising administering to an animal having a vascularized tumor a vascular endothelial cell killing amount of at least a first antibody, or antigen-binding region thereof, that binds to an aminophospholipid on the luminal surface of tumor vasculature.

5. The method of claim 4, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylethanolamine on the luminal surface of tumor vascular endothelial cells.

6. The method of claim 4, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylserine on the luminal surface of tumor vascular endothelial cells.

7. A method for arresting blood flow in tumor vasculature, comprising administering to an animal having a vascularized tumor a coagulation-inducing amount of at least a first antibody, or antigen-binding region thereof, that binds to an aminophospholipid on the luminal surface of tumor vasculature.

8. The method of claim 7, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylethanolamine on the luminal surface of tumor vascular endothelial cells.

9. The method of claim 7, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylserine on the luminal surface of tumor vascular endothelial cells.

10. A method for destroying tumor vasculature, comprising administering to an animal having a vascularized tumor a tumor-destructive amount of at least a first antibody, or antigen-binding region thereof, that binds to an aminophospholipid on the luminal surface of tumor vasculature.

11. The method of claim 10, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylethanolamine on the luminal surface of tumor vascular endothelial cells.

12. The method of claim 10, wherein said antibody, or antigen-binding region thereof, binds to phosphatidylserine on the luminal surface of tumor vascular endothelial cells.

13. A method for treating an animal having a vascularized tumor, comprising administering to said animal a therapeutically effective amount of a pharmaceutical composition comprising at least a first antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid on the luminal surface of blood vessels of the vascularized tumor.

14. The method of claim 13, wherein said pharmaceutical composition comprises at least a first antibody, or antigen-binding fragment thereof, that binds to phosphatidylethanolamine on the luminal surface of blood vessels of the vascularized tumor.

15. The method of claim 13, wherein said pharmaceutical composition comprises at least a first antibody, or antigen-binding fragment thereof, that binds to phosphatidylserine on the luminal surface of blood vessels of the vascularized tumor.

16. The method of claim 13, wherein said pharmaceutical composition comprises at least a first human antibody, or antigen-binding fragment thereof.

17. The method of claim 13, wherein said pharmaceutical composition comprises at least a first IgG or IgM antibody.

18. The method of claim 13, wherein said pharmaceutical composition comprises at least a first antigen binding region of an antibody.

19. The method of claim 13, wherein said pharmaceutical composition comprises at least a first monoclonal antibody or antigen-binding fragment thereof.

20. The method of claim 19, wherein said pharmaceutical composition comprises at least a first scFv, Fv, Fab', Fab or $F(ab')_2$ antigen-binding fragment of a monoclonal antibody.

21. The method of claim 19, wherein said pharmaceutical composition comprises at least a first human monoclonal antibody or antigen-binding fragment thereof.

22. The method of claim 19, wherein said pharmaceutical composition comprises at least a first humanized or part-human chimeric monoclonal antibody or antigen-binding fragment thereof.

23. The method of claim 19, wherein said pharmaceutical composition comprises at least the anti-phosphatidylserine monoclonal antibody 3SB9b, or antigen-binding fragment thereof.

24. The method of claim 19, wherein said pharmaceutical composition comprises at least a first monoclonal antibody, or antigen-binding fragment thereof, that is prepared by a preparative process comprising:

(a) preparing an anti-aminophospholipid antibody-producing cell; and

(b) obtaining an anti-aminophospholipid monoclonal antibody from said antibody-producing cell.

25. The method of claim 24, wherein said anti-aminophospholipid antibody-producing cell is obtained from a human patient having a disease associated with the production of anti-aminophospholipid antibodies.

26. The method of claim 24, wherein said anti-aminophospholipid antibody-producing cell is obtained by stimulating a mixed population of human peripheral blood lymphocytes with an immunogenically effective amount of an aminophospholipid sample.

27. The method of claim 24, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing an animal with an immunogenically effective amount of an aminophospholipid sample.

28. The method of claim 27, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing an animal via intrasplenic injection of an immunogenically effective amount of an aminophospholipid sample.

29. The method of claim 27, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing an animal by injection of an immunogenically effective amount of a *Salmonella*-coated aminophospholipid sample or an aminophospholipid micelle sample in combination with Freund's complete adjuvant.

30. The method of claim 27, wherein said anti-aminophospholipid antibody-producing cell is obtained by immunizing a transgenic mouse that comprises a human antibody library with an immunogenically effective amount of an aminophospholipid sample.

31. The method of claim 24, wherein said preparative process comprises:

(a) fusing said anti-aminophospholipid antibody-producing cell with an immortal cell to prepare a hybridoma that produces an anti-aminophospholipid monoclonal antibody; and

(b) obtaining an anti-aminophospholipid monoclonal antibody from said hybridoma.

32. The method of claim 24, wherein said preparative process comprises:

(a) immunizing an animal with an immunogenically effective amount of an aminophospholipid sample;

(b) preparing a collection of antibody-producing hybridomas from the immunized animal;

(c) selecting from the collection a hybridoma that produces an anti-aminophospholipid antibody; and

(d) culturing the selected hybridoma to provide the anti-aminophospholipid monoclonal antibody.

33. The method of claim 32, wherein the antigen binding region of the anti-aminophospholipid monoclonal antibody is operatively attached to a human antibody framework or constant region.

34. The method of claim 32, wherein the immunized animal is a transgenic mouse that comprises a human antibody library and wherein the anti-aminophospholipid monoclonal antibody is a human monoclonal antibody.

35. The method of claim 24, wherein said preparative process comprises:

(a) obtaining the anti-aminophospholipid antibody-encoding nucleic acids from said anti-aminophospholipid antibody-producing cell; and

(b) expressing said nucleic acids to obtain a recombinant anti-aminophospholipid monoclonal antibody.

36. The method of claim 24, wherein said preparative process comprises:

(a) immunizing an animal with an immunogenically effective amount of an aminophospholipid sample;

(b) preparing a combinatorial immunoglobulin phagemid library expressing RNA isolated from the spleen of the immunized animal;

(c) selecting from the phagemid library a clone that expresses an anti-aminophospholipid antibody; and

(d) expressing the anti-aminophospholipid antibody-encoding nucleic acids from said selected clone to provide a recombinant anti-aminophospholipid monoclonal antibody.

37. The method of claim 36, wherein the immunized animal is a transgenic mouse that comprises a human antibody library and wherein the recombinant anti-aminophospholipid monoclonal antibody is a recombinant human monoclonal antibody.

38. The method of claim 13, wherein said pharmaceutical composition comprises a dimer, trimer or multimer of an anti-aminophospholipid antibody or antigen-binding fragments thereof.

39. The method of claim 13, wherein at least a second antibody that binds to an aminophospholipid, or an antigen-binding fragment thereof, is administered to said animal.

40. The method of claim 13, wherein said pharmaceutical composition is administered to said animal via intravenous administration.

41. The method of claim 13, further comprising subjecting said animal to surgery or radiotherapy.

42. The method of claim 13, further comprising administering to said animal a therapeutically effective amount of at least a first anti-cancer agent.

43. The method of claim 42, wherein said at least a first anti-cancer agent is administered to said animal simultaneously with said at least a first antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid.

44. The method of claim 43, wherein said at least a first anti-cancer agent and said at least a first antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid are administered to said animal in a single pharmaceutical composition.

45. The method of claim 42, wherein said at least a first anti-cancer agent is administered to said animal sequentially to said at least a first antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid.

46. The method of claim 45, wherein said at least a first anti-cancer agent is administered to said animal subsequent to the administration of said at least a first antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid.

47. The method of claim 42, wherein said at least a first anti-cancer agent is a chemotherapeutic agent.

48. The method of claim 42, wherein said at least a first anti-cancer agent is a radiotherapeutic agent.

49. The method of claim 42, wherein said at least a first anti-cancer agent is an anti-angiogenic or apoptosis-inducing agent.

50. The method of claim 42, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed, surface-accessible or surface-localized component of a tumor cell,

tumor vasculature or tumor stroma; said antibody or fragment thereof operatively linked to a therapeutic agent.

51. The method of claim 50, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a cell surface antigen of a tumor cell.

52. The method of claim 51, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, selected from the group consisting of B3 (ATCC HB 10573), 260F9 (ATCC HB 8488), D612 (ATCC HB 9796) and KS1/4, said KS1/4 antibody obtained from a cell comprising the vector pGKC2310 (NRRL B-18356) or the vector pG2A52 (NRRL B-18357).

53. The method of claim 50, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a component of tumor stroma.

54. The method of claim 53, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a connective tissue component, a basement membrane component or a component of an activated platelet.

55. The method of claim 50, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed, surface-accessible or surface-localized component of intratumoral blood vessels of a vascularized tumor.

56. The method of claim 55, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a surface-expressed component of intratumoral blood vessels of a vascularized tumor.

57. The method of claim 56, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to an intratumoral vasculature cell surface receptor.

58. The method of claim 57, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to endoglin, a TGFβ receptor, E-selectin, P-selectin, VCAM-1, ICAM-1, PSMA, a VEGF/VPF receptor, an FGF receptor, a TIE, $\alpha_v\beta_3$ integrin, pleiotropin, endosialin or an MHC Class II protein.

59. The method of claim 58, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to endoglin, E-selectin or VCAM-1.

60. The method of claim 55, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a ligand or growth factor that binds to an intratumoral vasculature cell surface receptor.

61. The method of claim 60, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to VEGF/VPF, FGF, TGFβ, a ligand that binds to a TIE, a tumor-associated fibronectin isoform, scatter factor/hepatocyte growth factor (HGF), platelet factor 4 (PF4), PDGF or TIMP.

62. The method of claim 55, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a ligand:receptor complex or a growth factor:receptor complex, but that does not bind to the ligand or growth factor, or to the receptor, when the ligand or growth factor or the receptor is not in the ligand:receptor or growth factor:receptor complex.

63. The method of claim 55, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a cytokine-inducible component of intratumoral blood vessels of a vascularized tumor.

64. The method of claim 55, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an antibody, or antigen binding fragment thereof, that binds to a coagulant-inducible component of intratumoral blood vessels of a vascularized tumor.

65. The method of claim 50, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to a cytotoxic agent.

66. The method of claim 65, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to a plant-, fungus- or bacteria-derived toxin.

67. The method of claim 66, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to deglycosylated ricin A chain.

68. The method of claim 50, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to a coagulation factor or to an antibody, or antigen binding fragment thereof, that binds to a coagulation factor.

69. The method of claim 68, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to Tissue Factor or a Tissue Factor derivative, or to an antibody, or antigen binding fragment thereof, that binds to Tissue Factor or a Tissue Factor derivative.

70. The method of claim 69, wherein said at least a first anti-cancer agent is an antibody-therapeutic agent construct comprising an anti-tumor cell, anti-tumor vasculature or anti-tumor stroma antibody, or antigen binding fragment thereof, operatively linked to truncated Tissue Factor, or to an antibody, or antigen binding fragment thereof, that binds to truncated Tissue Factor.

71. The method of claim 13, wherein said animal has a vascularized tumor of at least about medium size.

72. The method of claim 71, wherein said animal has a large vascularized tumor.

73. The method of claim 13, wherein said animal is a mouse.

74. The method of claim 13, wherein said animal is a human patient.

75. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first antibody construct effective to specifically kill at least a portion of the tumor vascular endothelial cells; wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of tumor vascular endothelial cells.

76. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first

antibody construct effective to induce cell-mediated cytotoxicity of at least a portion of the tumor vascular endothelial cells, wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of tumor vascular endothelial cells.

77. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first antibody construct effective to induce complement-mediated lysis of at least a portion of the tumor vascular endothelial cells, wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of tumor vascular endothelial cells.

78. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first antibody construct effective to induce apoptosis in at least a portion of the tumor vascular endothelial cells; wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of tumor vascular endothelial cells.

79. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first antibody construct effective to specifically promote coagulation in tumor blood vessels; wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of tumor vascular endothelial cells.

80. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first antibody construct effective to specifically occlude or destroy tumor blood vessels, as opposed to normal blood vessels; wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of tumor vascular endothelial cells.

81. A method for treating an animal having a vascularized tumor, comprising administering to said animal at least a first pharmaceutical composition comprising an amount of at least a first antibody construct effective to induce tumor necrosis; wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid expressed on the luminal surface of blood vessels of the vascularized tumor.

82. The method of any one of claims 75 to 81, wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to phosphatidylethanolamine.

83. The method of any one of claims 75 to 81, wherein said first antibody construct is an antibody, or antigen-binding fragment thereof, that binds to phosphatidylserine.

84. A method for treating cancer, comprising administering to an animal having a vascularized tumor a therapeutically effective amount of a pharmaceutical composition comprising at least a first naked antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid on the luminal surface of intratumoral blood vessels of the vascularized tumor.

85. The method of claim 84, wherein said naked antibody is an antibody, or antigen-binding fragment thereof, that binds to phosphatidylethanolamine.

86. The method of claim 84, wherein said naked antibody is an antibody, or antigen-binding fragment thereof, that binds to phosphatidylserine.

87. A method for treating cancer, comprising administering to an animal having a vascularized tumor a necrosis-inducing amount of a pharmaceutical composition comprising at least a first unconjugated antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid on the luminal surface of blood vessels of the vascularized tumor.

88. The method of claim 87, wherein said unconjugated antibody is an unconjugated antibody, or antigen-binding fragment thereof, that binds to phosphatidylethanolamine.

89. The method of claim 87, wherein said unconjugated antibody is an unconjugated antibody, or antigen-binding fragment thereof, that binds to phosphatidylserine.

90. A method for treating a patient with cancer, comprising selecting a suitable patient having a vascularized tumor and administering to said patient a therapeutically effective amount of at least a first pharmaceutical composition comprising at least a first antibody, or antigen-binding fragment thereof, that binds to an aminophospholipid on the luminal surface of intratumoral blood vessels of the vascularized tumor.

91. A therapeutic kit comprising, in at least a first suitable container, a biologically effective amount of at least a first antibody, or an antigen-binding fragment thereof, that binds to an aminophospholipid; and a biologically effective amount of at least a first anti-cancer agent.

92. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof is an anti-phosphatidylethanolamine antibody or fragment thereof.

93. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof is an anti-phosphatidylserine antibody or fragment thereof.

94. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof is a human or humanized antibody or fragment thereof.

95. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof is a monoclonal antibody or fragment thereof.

96. The therapeutic kit of claim 91, wherein said at least a first anti-cancer agent is a chemotherapeutic agent, radiotherapeutic agent, anti-angiogenic or apoptosis-inducing agent.

97. The therapeutic kit of claim 91, wherein said at least a first anti-cancer agent is an antibody construct comprising a second antibody, or antigen binding fragment thereof, that binds to a surface-expressed, surface-accessible or surface-localized component of a tumor cell, tumor vasculature or tumor stroma; said second antibody or fragment thereof operatively linked to a therapeutic agent.

98. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof and said at least a first anti-cancer agent are comprised within a single container.

99. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof and said at least a first anti-cancer agent are comprised within distinct containers.

100. The therapeutic kit of claim 91, wherein said at least a first antibody or fragment thereof and said at least a first anti-cancer agent are formulated for intravenous administration.

101. A medicinal cocktail, comprising a combined effective amount of at least a first anti-cancer agent and at least a first antibody, or an antigen-binding fragment thereof, that binds to an aminophospholipid.

ABSTRACT

Disclosed are the surprising discoveries that aminophospholipids, such as phosphatidylserine and phosphatidylethanolamine, are specific markers accessible on the luminal surface of tumor blood vessels, and that the administration of an anti-aminophospholipid antibody alone is sufficient to induce thrombosis, tumor necrosis and tumor regression *in vivo*. This invention therefore provides anti-aminophospholipid antibody-based methods and compositions for use in the specific destruction of tumor blood vessels and in the treatment of solid tumors. Although various antibody conjugates and combinations are thus provided, the use of naked, or unconjugated, anti-phosphatidylserine antibodies is a particularly important aspect of the invention, due to simplicity and effectiveness of the approach.